AMENDMENTS TO THE CLAIMS

Please amend claims 1-4, 8, 20, 21, 23, 26, 28, 29, 33-35, 37, 43, and 45.

The listing of claims will replace all prior versions, and listings of claims in the application.

Listing of Claims

1. (Currently Amended) A method for detecting a colon cell proliferative disorder in a human subject comprising:

a) obtaining from the subject a biological sample comprising genomic DNA from blood plasma, blood serum, whole blood, or ~~isolated blood~~ colon cells; ~~or~~

b) contacting the genomic DNA with at least one reagent that distinguishes between methylated and non-methylated CpG dinucleotide sequences within ALX4 gene sequence (SEQ ID NO:5) to determine CpG methylation status; and

c) comparing the CpG methylation status of ALX4 gene in the sample of a) with the CpG methylation status of ALX4 gene of a biological sample comprising genomic DNA from blood plasma, blood serum, from blood plasma, blood serum, whole blood, or colon cells from a human subject not having a colon cell proliferative disorder, wherein a difference ~~in~~ between the CpG methylation status of ALX4 gene from the sample of a) and the CpG methylation status of ALX4 gene from the biological sample from a human subject not having a colon cell proliferative disorder is ~~indicative of a colon cell proliferative disorder~~ associable with development of colorectal carcinoma and other colorectal cell proliferative disorder in the subject.

2. (Currently Amended) The method of claim 1, wherein the CpG dinucleotide sequence comprises at least 16 contiguous nucleotides of ~~the~~ ALX 4 (SEQ ID NO: 5) gene sequence.

3. (Currently Amended) A method for detecting a colon cell proliferative disorder in a human subject comprising:

a) obtaining from the subject a biological sample comprising genomic DNA from blood plasma, blood serum, from blood plasma, blood serum, whole blood, or colon cells;

b) extracting the genomic DNA;

c) determining the expression levels of ALX 4 gene (SEQ ID NO:5) or gene sequences thereof, in [[a]] the sample from the subject ~~comprising colon cells, colon fluid, stool, or colon tissue~~; and

d) comparing ~~the~~ ALX 4 gene (SEQ ID NO:5) expression level in the sample with ~~the~~ ALX 4 gene (SEQ ID NO: 5) expression level of a biological sample comprising genomic DNA from blood plasma, blood serum, from blood plasma, blood serum, whole blood, or colon cells from a human subject not having a colon cell proliferative disorder, wherein reduced expression of the ALX 4 gene (SEQ ID NO:5) in the sample as compared with the sample from the subject not having a colon cell proliferative disorder ~~is indicative of a colon cell proliferative disorder~~ is associable with the development of colorectal carcinoma and other colorectal cell proliferative disorder in the subject.

4. (Currently Amended) The method of claim 3, wherein the expression level is determined by detecting the presence, absence or level of mRNA transcribed from ~~the~~ ALX 4 gene (SEQ ID NO:5).

5. (Previously Presented) The method of claim 3, wherein the expression level is determined by detecting the presence, absence or level of a polypeptide encoded by the ALX 4 gene (SEQ ID NO:5).

6. (Previously Presented) The method of claim 5, wherein the polypeptide is detected by a method selected from the group consisting of immunoassay, ELISA immunoassay, radioimmunoassay and antibody binding.

7. (Previously Presented) The method of claim 3, wherein the expression level is determined by detecting the presence or absence of CpG methylation within the gene or gene sequence thereof.

8. (Currently Amended) A method for detecting a colon cell proliferative disorder in a human subject comprising:

a) obtaining, from the subject, a biological sample comprising genomic DNA from blood plasma, blood serum, whole blood, ~~isolated blood cells~~, or colon cells~~, colon fluid, stool, or colon tissue~~;

b) contacting the genomic DNA, or a fragment thereof with at least one reagent that distinguishes between methylated and non-methylated CpG dinucleotide sequences within at least one target region wherein the at least one target region comprises, or hybridizes under stringent conditions to 9 contiguous nucleotides of ALX 4 gene sequence (SEQ ID NO: 5), and the contiguous nucleotides comprise at least one CpG dinucleotide sequence to determine CpG methylation status; and

c) comparing the CpG methylation status of ALX4 gene in the sample of a) with the CpG methylation status of ALX4 gene of a biological sample comprising genomic DNA from blood plasma, blood serum, from blood plasma, blood serum, whole blood, or colon cells from a human subject not having a colon cell proliferative disorder, wherein a difference ~~in~~ between the CpG methylation status of ALX4 gene from the sample of a) and the CpG methylation status of ALX4 gene from the biological sample from a human subject not having a colon cell proliferative disorder is ~~indicative of a colon cell proliferative disorder~~ associable with the development of colorectal carcinoma and other colorectal cell proliferative disorder in the subject.

9. (Canceled)

10. (Previously Presented) The method of claim 8, wherein the colon cell proliferative disorder is colorectal carcinoma.

11. (Previously Presented) The method of claim 8, wherein the colon cell proliferative disorder is colon adenoma.

12-19. (Canceled)

20. (Currently Amended) A method for detecting a colon cell proliferative disorder in a human subject comprising:

a) obtaining, from the subject, a biological sample comprising genomic DNA from blood plasma, blood serum, whole blood, ~~isolated blood cells~~, or colon cells~~, colon fluid, stool, or colon tissue~~;

b) contacting the genomic DNA, or a fragment thereof, with at least one reagent that distinguishes between methylated and non-methylated CpG dinucleotide sequences to determine CpG methylation status; ~~and~~

c) amplifying at least one target sequence of the DNA with at least one primer pair, wherein the target sequence comprises, or hybridizes under stringent conditions to an at least 16 contiguous nucleotide sequence of a sequence selected from the group consisting of SEQ ID NOS: 5, 312, 313, 428, and 429 and a complement thereof, wherein the contiguous nucleotide sequence comprises at least one CpG dinucleotide sequence; and

d) comparing the CpG methylation status of ALX4 gene in the sample of a) with the CpG methylation status of ALX4 gene of a biological sample comprising genomic DNA from blood plasma, blood serum, from blood plasma, blood serum, whole blood, or colon cells from a human subject not having a colon cell proliferative disorder, wherein a difference between the CpG methylation status of ALX4 gene from the sample of a) and the CpG methylation status of ALX4 gene from the biological sample from a human subject not having a colon cell proliferative disorder is associable with the development of colorectal carcinoma and other colorectal cell proliferative disorder in the subject.

21. (Currently Amended) The method of claim 8, ~~comprising~~ wherein the CpG methylation status is determined by:

a) treating the genomic DNA, or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated in the 5-position thereof to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties;

b) contacting the treated genomic DNA of a),or the treated fragment thereof, within ALX4 gene sequence (SEQ ID NO:5) with an amplification enzyme and at least two primers ~~comprising~~, that [[is]] are complementary to, or hybridize[[s]] under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS: 312, 313, 428 and 429, and complements thereof, wherein the treated genomic DNA or the fragment thereof is either amplified to produce at least one amplificate, or is not amplified; and

c) determining, based on a presence or absence of, or on a property of ~~said~~ the amplificate, the methylation state of at least one CpG dinucleotide of a sequence selected within ALX4 gene sequence SEQ ID NO: 5, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotides.; ~~and~~

~~d) comparing the CpG methylation status in the sample with the CpG methylation status from a subject not having a colon cell proliferative disorder, wherein a difference in the CpG methylation status is indicative of a colon cell proliferative disorder.~~

22. (Previously Presented) The method of claim 21, wherein treating the genomic DNA, or the fragment thereof, comprises use of at least one reagent selected from the group consisting of bisulfite, hydrogen sulfite, and disulfite.

23. (Currently Amended) The method of claim 21, wherein the contacting in b) comprises use of at least one ~~method selected from the group consisting of use~~ of: a heat resistant DNA polymerase as the amplification enzyme; ~~use of~~ a polymerase lacking 5'-3' exonuclease

activity; ~~use of~~ a polymerase chain reaction (PCR); ~~and~~ or generation of an amplificate nucleic acid molecule carrying a detectable label.

24. (Previously Presented) The method of claim 23, wherein the detectable label is selected from the label group consisting of fluorescent labels; radionuclides or radio labels; amplificate mass labels detectable in a mass spectrometer; detachable amplificate fragment mass labels detectable in a mass spectrometer; amplificate, and detachable amplificate fragment mass labels having a single-positive or single-negative net charge detectable in a mass spectrometer; and combinations thereof.

25. (Original) The method of claim 21, wherein the biological sample obtained from the subject is selected from the group consisting of cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids, stool, colonic effluent, urine, blood plasma, blood serum, whole blood, isolated blood cells, cells isolated from the blood and combinations thereof.

26. (Currently Amended) The method of claim 21, wherein the at least 9 contiguous nucleotides ~~of b)~~ suppresses amplification of the nucleic acid to which ~~it is~~ they are hybridized.

27. (Previously Presented) The method of claim 26, wherein the sequence of said nucleic acid molecules is selected from the group consisting of SEQ ID NOS: 3030, 3035, 3046, 3058, 3062, 3067, 3070, 3074, 3077, 3079, 3082, 3087, 3095, 3099, 3102, 3106, 3112, 3120, 3125, 3129, 3132, 3141, 3143, 3154, 3156, and 3158.

28. (Currently Amended) The method of claim 26, ~~wherein the~~ further comprising a nucleic acid molecule or a peptide nucleic acid molecule ~~is~~ modified at the 5' end ~~thereof~~ to preclude degradation by an enzyme having 5' -3' exonuclease activity.

29. (Currently Amended) The method of claim 26, ~~wherein the~~ further comprising a nucleic acid molecule or a peptide nucleic acid molecule ~~is~~ lacking a 3' hydroxyl group.

30. (Original) The method of claim 26, wherein the amplification enzyme is a polymerase lacking 5' -3' exonuclease activity.

31. (Previously Presented) The method of claim 21, wherein the determining in c) comprises hybridization of at least one nucleic acid molecule or peptide nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:312, 313, 428 and 429, and complements thereof, thereby producing hybridized nucleic acid molecules or peptide nucleic acid molecules.

32. (Previously Presented) The method of claim 31, wherein the nucleic acid is selected from the group consisting of SEQ ID NOS:3030, 3035, 3046, 3058, 3062, 3067, 3070, 3074, 3077, 3079, 3082, 3087, 3095, 3099, 3102, 3106, 3112, 3120, 3125, 3129, 3132, 3141, 3143, 3154, 3156, and 3158.

33. (Currently Amended) The method of claim 31, wherein ~~the~~ at least one of the hybridized nucleic acid molecules or peptide nucleic acid molecules is bound to a solid phase.

34. (Currently Amended) The method of claim 31, wherein the hybridized nucleic acid molecules or peptide nucleic acid molecules are bound to a solid phase in the form of a nucleic acid or peptide nucleic acid array selected from the array group consisting of substantially linear array, substantially hexagonal array, substantially rectangular array, and combinations thereof.

35. (Currently Amended) The method of claim 31, further comprising extending at least one of the hybridized nucleic acid molecules by at least one nucleotide base.

36. (Previously Presented) The method of claim 21, wherein the determining in c),

comprises sequencing of the amplificate.

37. (Currently Amended) The method of claim 21, wherein the primers of [[a)]] b) are methylation-specific primers.

38. (Previously presented) The method of claim 37, wherein the sequence of said methylation-specific primers is selected from the group consisting of SEQ ID NOS:3028, 3032, 3033, 3036, 3037, 3038, 3039, 3041, 3042, 3043, 3044, 3047, 3048, 3049, 3052, 3055, 3059, 3061, 3064, 3065, 3068, 3069, 3071, 3072, 3075, 3076, 3080, 3083, 3084, 3085, 3086, 3091, 3093, 3096, 3097, 3100, 3104, 3109, 3110, 3113, 3115, 3117, 3118, 3123, 3126, 3127, 3130, 3134, 3135, 3136, 3138, 3139, 3144, 3146, 3147, 3149, 3150, 3155, 3029, 3031, 3034, 3040, 3045, 3050, 3051, 3053, 3054, 3056, 3057, 3060, 3063, 3066, 3073, 3078, 3081, 3088, 3089, 3090, 3092, 3094, 3098, 3101, 3103, 3105, 3107, 3108, 3111, 3114, 3116, 3119, 3121, 3122, 3124, 3128, 3131, 3133, 3137, 3140, 3142, 3145, 3148, 3151, 3152, 3153, and 3157.

39. (Previously Presented) The method of claim 21, wherein the primers are oligonucleotides comprising one or more CpG; TpG or CpA dinucleotides.

40. (Canceled)

41. (Previously Presented) The method of claim 21, wherein the primers of b) are oligonucleotides comprising one or more CpG; TpG or CpA dinucleotides used for amplification and further comprising hybridizing at least one detectably labeled nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:312, 313, 428 and 429.

42. (Previously Presented) The method of claim 21, comprising in b) the use of at least one nucleic acid molecule or peptide nucleic acid molecule comprising a contiguous

sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:312, 313, 428 and 429, and complements thereof, wherein said nucleic acid molecule or peptide nucleic acid molecule suppresses amplification of the nucleic acid to which it is hybridized, and further comprising hybridizing at least one detectably labeled nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:312, 313, 428 and 429, and complements thereof.

43. (Currently Amended) The method of claim 39, wherein the primers ~~oligonucleotides of c)~~ of b) are selected from the group consisting SEQ ID NOS: 3028, 3032, 3033, 3036, 3037, 3038, 3039, 3041, 3042, 3043, 3044, 3047, 3048, 3049, 3052, 3055, 3059, 3061, 3064, 3065, 3068, 3069, 3071, 3072, 3075, 3076, 3080, 3083, 3084, 3085, 3086, 3091, 3093, 3096, 3097, 3100, 3104, 3109, 3110, 3113, 3115, 3117, 3118, 3123, 3126, 3127, 3130, 3134, 3135, 3136, 3138, 3139, 3144, 3146, 3147, 3149, 3150, 3155, 3029, 3031, 3034, 3040, 3045, 3050, 3051, 3053, 3054, 3056, 3057, 3060, 3063, 3066, 3073, 3078, 3081, 3088, 3089, 3090, 3092, 3094, 3098, 3101, 3103, 3105, 3107, 3108, 3111, 3114, 3116, 3119, 3121, 3122, 3124, 3128, 3131, 3133, 3137, 3140, 3142, 3145, 3148, 3151, 3152, 3153, and 3157.

44. (Canceled)

45. (Currently Amended) A method for detecting colon cell proliferative disorders in a human subject comprising:

a) obtaining, from the subject, a biological sample comprising genomic DNA from blood plasma, blood serum, whole blood, isolated blood cells, colon cells, colon fluid, stool, or colon tissue;

b) extracting, or otherwise isolating the genomic DNA;

c) contacting the genomic DNA ~~of b)~~, or a fragment thereof, comprising at least 16

contiguous nucleotides of ALX4 (SEQ ID NO: 5) and sequences that hybridize under stringent conditions thereto, with one or more methylation-sensitive restriction enzymes, wherein the genomic DNA is either cleaved thereby to produce cleavage fragments, or not cleaved thereby;

d) determining the CpG methylation status of SEQ ID NO:5, or an average, or a value reflecting an average methylation status of a plurality of CpG dinucleotides of target CpG dinucleotide sequences within SEQ ID NO:5; and

e) comparing the CpG methylation status of ALX4 gene in the sample of a) with the CpG methylation status of ALX4 gene of a biological sample comprising genomic DNA from blood plasma, blood serum, from blood plasma, blood serum, whole blood, or colon cells from a human subject not having a colon cell proliferative disorder, wherein a difference ~~in~~ between the CpG methylation status of ALX4 gene from the sample of a) and the CpG methylation status of ALX4 gene from the biological sample from a human subject not having a colon cell proliferative disorder is ~~indicative of a colon cell proliferative disorder~~ associable with the development of colorectal carcinoma and other colorectal cell proliferative disorder in the subject.

46. (Withdrawn) A treated nucleic acid derived from genomic SEQ ID NOS:5, wherein the treatment is suitable to convert at least one unmethylated cytosine base of the genomic DNA sequence to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization.

47. (Withdrawn) A nucleic acid, comprising at least 16 contiguous nucleotides of a treated genomic DNA sequence selected from the group consisting of SEQ ID NOS:312, 313, 428 and 429, and sequences complementary thereto, wherein the treatment is suitable to convert at least one unmethylated cytosine base of the genomic DNA sequence to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization.

48. (Withdrawn) The nucleic acid of claim 46, wherein the contiguous base sequence comprises at least one CpG, TpG or CpA dinucleotide sequence.

49. (Withdrawn) The nucleic acid of claim 46, wherein the treatment comprises use of a reagent selected from the group consisting of bisulfite, hydrogen sulfite, disulfite, and combinations thereof.

50. (Withdrawn) An oligomer, comprising a sequence of at least 9 contiguous nucleotides that is complementary to, or hybridizes under stringent conditions to a treated genomic DNA sequence selected from the group consisting of SEQ ID NOS:312, 313, 428 and 429.

51. (Withdrawn) The oligomer of Claim 49, comprising at least one CpG, CpA or TpG dinucleotide sequence.

52. (Canceled)

53. (Withdrawn) A kit useful for detecting, or for detecting and differentiating between or among colon cell proliferative disorders of a subject, comprising:

-a methylation-sensitive restriction enzyme; and

-at least one nucleic acid molecule or peptide nucleic acid molecule, comprising a contiguous sequence of at least 16 nucleotides that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:5, and complements thereof.

54. (Canceled)

55. (Withdrawn) A kit useful for detecting, or for detecting and differentiating between or among colon cell proliferative disorders of a subject, comprising:

-a bisulfite reagent; and

-at least one nucleic acid molecule or peptide nucleic acid molecule, comprising a contiguous sequence of at least 16 nucleotides that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:312, 312, 428 and 429, and complements thereof.

56. (Canceled)

57. (Withdrawn) The kit of claim 54, further comprising standard reagents for performing a methylation assay selected from the group consisting of MS-SNuPE, MSP, MethyLight, HeavyMethyl, COBRA, nucleic acid sequencing, and combinations thereof.

58. (Withdrawn) The kit of claim 52, wherein the length of the contiguous nucleotide sequence is selected from the group consisting of at least 17, at least 18, at least 20, at least 22, at least 23, at least 25, at least 27, at least 30, and at least 35 nucleotides.

59. (Withdrawn) The kit of claim 52, wherein the length of the contiguous nucleotide sequence is at least 18 nucleotides.

60. (Previously Presented) The method of claim 1, wherein the detection of the colon cell proliferative disorder has a sensitivity of greater than or equal to 80% and a specificity of greater than or equal to 80%.